

1-19-00 A  
**CONTINUING PATENT APPLICATION TRANSMITTAL**  
(for Continuing Applications  
under 37 C.F.R. §1.53(b))

Attorney Docket No. 68019

First Named Inventor or  
Application Identifier: Bedford

01/18/00  
jc525 U.S. PRO  
BOX PATENT APPLICATION

Commissioner of Patents and Trademarks  
ATTENTION: Assistant Commissioner  
for Patents  
Washington, D.C. 20231

Sir:

This is a request under 37 C.F.R.  
§1.53(b) for filing a:

- (X) Continuation application,  
( ) Divisional application,  
( ) Continuation-in-Part application,

of pending prior application number PCT/EP98/04440,

filed on 16 July 1998 of Bedford et al.

(Date)

(Inventor(s))

for USE OF ENZYME FOR THE MANUFACTURE OF AN AGENT FOR CONTROLLING  
(Title)

BACTERIAL INFECTION

1. ( ) This is a continuation or divisional application. Enclosed is a copy of the prior application as originally filed, including specification, claims, drawings, and oath or declaration.

- or -

- (X) Enclosed is a patent application (for continuation, divisional, or continuation-in-part applications) containing:

(X) 21 pages of the specification (including claims).

(X) 13 sheets of drawings (Figs. 1-3) (X) Formal ( ) Informal.

2. (X) Amend the specification by inserting before the first line the sentence: --This is a [X] continuation, [ ] division, [ ] continuation-in-part, of prior application number PCT/EP98/04440, filed 16 July 1998 Designated the U.S., which is hereby incorporated herein by reference in its entirety.-- The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under paragraph 3 below, is considered as being part of the disclosure of the accompanying application, and is hereby incorporated by reference therein.

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3. (X) A copy of the executed oath or declaration filed in the prior nonprovisional application is enclosed.
4. (X) Inventorship:
  - ( ) A newly-executed oath or declaration and power of attorney is enclosed (for continuation-in-part applications, or for continuation or divisional applications naming an inventor not named in the prior application) (§1.63(a), (d)(5) and (e)).
  - ( ) Because this application is being filed by fewer than all of the inventors named in the prior application, delete the following inventor(s) named in the prior nonprovisional application (37 C.F.R. §1.63(d)(1)(2)):  
\_\_\_\_\_  
\_\_\_\_\_.
  - (x) The names of persons believed to be the actual inventors are set forth in the enclosed unexecuted oath or declaration and power of attorney (§1.41(a) and §1.53(b)).
5. ( ) Assignment(s) of the invention to \_\_\_\_\_, and cover sheet are enclosed.
  - ( ) A check in the amount of \$\_\_\_\_\_ to cover the fee for recording the assignment(s) is enclosed.
6. ( ) The prior application is assigned of record to \_\_\_\_\_.
7. ( ) Small Entity Status (37 C.F.R. §1.28(a)(2)):
  - ( ) A statement of status as a small entity is enclosed.
  - ( ) A statement of status as a small entity was filed in the prior application, and small entity status is still proper and desired in this new nonprovisional application.
  - ( ) Status as a small entity is no longer claimed.
8. ( ) A 37 C.F.R. §3.73(b) statement is enclosed (where an assignee seeks to take action in a matter before the Patent Office).
9. ( ) A preliminary amendment is enclosed.
10. ( ) Drawings:
  - ( ) Transfer the drawings from the prior application to this application and abandon said prior application as of the filing date accorded this application. A duplicate copy of this sheet is enclosed for filing in the prior application file. (May be used only if signed by person authorized by §1.138 and before payment of base issue fee.)

( ) New formal drawings are enclosed.

( ) Informal drawings are enclosed.

11. (X) A separate written request under 37 C.F.R. §1.136(a)(3), which is a general authorization to treat any concurrent or future reply requiring a petition for an extension of time under 37 C.F.R. §1.136(a) for its timely submission as incorporating a petition for an extension of time for the appropriate length of time, is enclosed.

12. ( ) An Information Disclosure Statement is enclosed.

( ) A Form PTO-1449 is enclosed.

( ) \_\_\_\_\_ References (copies) listed on the Form PTO-1449 are enclosed.

13. ( ) A MicroFiche Computer Program (Appendix) is enclosed.

14. (X) A Return Receipt Postcard is enclosed (MPEP §503).

15. ( ) A Nucleotide and/or Amino Acid Sequence Submission is enclosed.

( ) A Computer Readable Copy is enclosed.

( ) A Paper Copy (Identical to Computer Copy) is enclosed.

( ) A Statement Verifying Identity of above Copies is enclosed.

16. (X) Priority of application number GB/9715214.4 filed on 18 July 1997 in Great Britian is claimed under 35 U.S.C. §119.

( ) The certified copy of the priority document has been filed in prior application number \_\_\_\_/\_\_\_\_, filed \_\_\_\_\_.

( ) A certified copy of the priority document is enclosed.

17. (X) Power of Attorney:

( ) The power of attorney in the prior application is to:

( ) \_\_\_\_\_ Reg. No. \_\_\_\_\_,  
FITCH, EVEN, TABIN, & FLANNERY  
Suite 1600  
120 South LaSalle Street  
Chicago, Illinois 60603-3406  
and other members of the firm.

( ) Customer Number 22242.

( ) The power appears in the original papers in the prior application.

( ) Since the power does not appear in the original papers in the prior application, a copy of the power in the prior application is enclosed.

18. ( ) Cancel in this application original claims \_\_\_\_\_ of the prior application before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)

19. (X) The filing fee is calculated below:

| Fee Calculation for Claims as Filed in the Prior Application,<br>Less Any Claims Cancelled by Amendment |           |                 |
|---|-----------|-----------------|
| (X) Basic Utility Fee   | \$ 690.00 | \$ _____        |
| • ( ) Independent Claims _____ - 3 = _____ x \$ 78.00 = \$ _____  |           |                 |
| • ( ) Total Claims _____ - 20 = _____ x \$ 18.00 = \$ _____   |           |                 |
| • ( ) Fee for Multiply Dependent Claims   | \$260.00  | \$ _____        |
| or  |           |                 |
| ( ) Basic Design Fee  | \$ 310.00 | \$ _____        |
| Total of above Calculations   |           | \$ <u>DEFER</u> |
| Reduction by 50% for Filing by Small Entity   |           | \$ _____        |
| Total   |           | \$ <u>DEFER</u> |

20. ( ) A check in the amount of \$ \_\_\_\_\_ is enclosed.

21. (X) The payment of the Filing Fee is to be deferred until the Declaration is filed. Do not charge our Deposit Account.

22. (X) The Commissioner is hereby authorized to charge any fees which may be required under 37 C.F.R. §§1.16 and 1.17 and are not paid herewith, or credit any overpayment, to Deposit Account Number 06-1135. A duplicate copy of this request is enclosed.

23. ( ) Also enclosed:

24. (X) Address all future communications to Customer Number 22242.



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JAN 18, 2000  
(Date)

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Registration No. 35,234  
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( ) Filed under §1.34(a)

- 1 -

Finnfeeds International Ltd

USE OF AN ENZYME FOR THE MANUFACTURE OF AN AGENT FOR  
CONTROLLING BACTERIAL INFECTION

The present invention is directed to the use of an enzyme for the manufacture of an agent for the treatment and/or prophylaxis of a bacterial infection.

The farming of many different types of animals is important throughout the world for the production of food for human consumption. When the animals are reared, they come into contact with a variety of infection-causing bacteria, such as *Campylobacter* and *Salmonella*. In some cases these bacteria may spread directly from animals to humans (zoonosis). Accordingly, it is necessary from an economic, environmental and health perspective that such bacterial infection is prevented or eradicated in the animal prior to human consumption to prevent the spread of the disease to humans.

The domestic animal of particular, but not exclusive, concern with regard to zoonosis is the chicken. *Campylobacter* and *Salmonella* are particularly prevalent in the chicken. The bacteria are transmitted to the bird in a variety of ways, including through feed, water, litter and vermin. The bacteria initially infect the caecae of the chicken. The disease then progresses to the small intestine where infestation may cause loss of weight in the bird. A particular problem with the chicken

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is that it is almost impossible to eviscerate in a sterile manner with the result that bacteria inhabiting the intestines will invariably be transmitted to the saleable carcass. Accordingly the potential for zoonosis is great, unless the carcass is handled or cooked properly. The cost of human infection caused by eating improperly treated chicken is significant in terms of both time and lives.

Accordingly, presently there is a demand for improved methods of reducing bacterial infection in animals such as the chicken, in particular those intended for human consumption.

Various solutions to the problem of bacterial infection have been proposed. Current methods of control include the application of antibiotics, feed sterilisation and careful and controlled handling and cooking of the carcass after slaughter. Feed sterilisation has proved ineffective in the absence of a sterile rearing environment (which is impractical) whilst controlled handling and cooking cannot be relied upon in every instance. The application of antibiotics has proved unpopular with consumer groups wishing to reduce the quantity of potentially harmful chemicals in food. The use of antibiotics has the additional problem that if they are not introduced into the animal in a properly controlled manner, antibiotic-resistant strains of bacteria can be created, making such infections more difficult to treat in the future. The prophylactic use of

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antibiotics in animal feed has thus been regulated in some countries (notably Sweden and Finland) effectively reducing the available methods of control. Indeed no single method provides a barrier which completely prevents bacteria being transferred from the animal to humans.

As an alternative to the above methods it has been proposed in *Poultry Science*, 1994 73:402-407, to introduce flora into chickens to compete with the bacteria causing the infection. Such mucosal competitive exclusion flora (MCE) were found to reduce the level of *Campylobacter jejuni* infection in chickens. However, the competitive exclusion treatment is not found to be consistently effective, its efficacy varying from animal to animal.

JP-A-81-73055 discloses animal feeds intended to prevent contamination with *Salmonella*. The feeds are indicated to contain partially decomposed mannan in the form of mannose polysaccharides. These are produced by degradation of mannan with an enzyme, produced by micro-organisms. The resulting feed was found to be moderately effective against *Salmonella* in chickens, but is not effective against *Campylobacter*.

US-A-5 124 262 discloses a mannose isomerase enzyme used for converting fructose to mannose. The mannose thus produced is taught to be useful in feeds, for inhibiting the growth of *Salmonella* in chickens.

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In Bamboo J. 1993, pp. 29-35, xylan prepared from steamed bamboo grass is mentioned as inhibiting the growth of various human intestinal bacteria. In particular the xylan is indicated to be effective against *Salmonella*. However, the inhibition effect is reversed after a period of 24 hours.

The above methods have proved more desirable from an environmental and health point of view, than the administration of antibiotics. However, none have proved effective enough to be commercially viable.

WO 93/01800 discloses the use of a protease for the preparation of a medicament effective against intestinal pathogens in animals. The pathogens of interest include *Campylobacter*. However, there is no mention of enzymes other than proteases being useful in controlling animal pathogens.

EP-A-0 681 787 discloses use of a carbohydrase or protease for the manufacture of an agent for the treatment of *Coccidiosis*. However, *Coccidiosis* in chickens is caused by protozoal oocytes. The document does not indicate how bacterial pathogens in chickens, or other animals, can be controlled.

Accordingly, one object of the present invention is to provide an agent which can be used for controlling bacterial infection that is more effective than the

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presently available agents, and in particular than those described in the prior art acknowledged above. A further object of the present invention is to provide an agent which can be used for controlling bacterial infection that is less harmful to the environment, less expensive than the presently available agents, and has advantages for human health.

Accordingly, the present invention provides the use of a xylanase or a cellulase for the manufacture of an agent for the treatment and/or prophylaxis of bacterial infection in an animal caused by *Salmonella*, *Campylobacter* or *Clostridium perfringens*.

A preferred cellulase is  $\beta$ -glucanase.

Figure 1 shows the effect of three diets on *Campylobacter* colonisation in 12-day old chicks. Three different dilution levels of the initial stock solution of *Campylobacter* were used to introduce the pathogen to the chicks. The results are presented as mean scores of positive caecae and represent the combined results of two flocks A and B comprising a total of 108 birds (12 per dilution for each of the three diets).

Figure 2 shows the results of Figure 1, but for flock A alone.

Figure 3 shows the results of Figure 1, but for flock B alone.

Figure 4 shows similar results to Figure 1, but at three alternative dilution levels of the initial *Campylobacter* stock solution. The results are presented as mean counts  $\log_{10}$ CFU(Colony Forming Units)/ml.

Figure 5 shows the results of Figure 4, but for flock A alone.

Figure 6 shows the results of Figure 4, but for flock B alone.

Figure 7 shows the effect of three diets on *Campylobacter* colonisation of the small intestine and caecae of 17-day old chicks. The results are presented as mean counts  $\log_{10}$ CFU/ml, and represent the combined results of two flocks A and B comprising a total of 72 birds (12 per treatment) for each of the three diets.

Figure 8 shows the results of Figure 7, but for flock A alone.

Figure 9 shows the results of Figure 7, but for flock B alone.

Figure 10 shows a comparison of the weight of 1, 5, 12, 19, 25 and 33-day old chicks (20 in total) dosed with *Campylobacter jejuni*, and similar chicks (25 in total) which have not been dosed, the chicks all being fed a wheat-based diet.

Figure 11 shows similar results to those of Figure 10, but for chicks on a wheat plus xylanase diet.

Figure 12 shows similar results to those of Figure 10, but for chicks on a maize-based diet.

Figure 13 shows the effect of two different diets (wheat and wheat plus xylanase) on *Salmonella enteritidis* colonisation in 14 day old chicks. The results are presented as mean counts  $\log_{10}$ CFU/ml. Tests were carried out on two flocks, A and B, comprising a total of 48 birds, 24 per diet.

The advantage of using feeds containing a xylanase or a cellulase for rearing animals is that the amount of antimicrobial drugs which have previously been routinely incorporated into their diet can be reduced, or in some cases omitted entirely. This enables considerable economic savings to be achieved in view of the relative expense of antibiotics. In countries where such drugs are banned, it represents a totally new approach to the control of bacterial diseases.

When omitting antibiotics from an animal's diet there are several potential further benefits. It has previously been necessary to withdraw antibiotics from the animal's diet for a certain time prior to slaughter. This ensures that the meat is relatively free from such drugs and thus fit for human consumption. In contrast, if antibiotics

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are entirely omitted from an animal's diet, as may be achieved with the present invention, then the animal can be slaughtered at any age rather than after a certain withdrawal period. This affords the farmer improved flexibility and removes the risk of animals becoming infected shortly prior to slaughter. Further, meat and eggs can be guaranteed free of antibiotics. Such meat and eggs have a market advantage as compared to products which cannot support such a guarantee.

Even if the enzyme added to the animal's diet only enables the level of inclusion of antibiotics to be reduced, then the overall cost of controlling bacterial infection will be reduced. Synergy or potentiation may extend the useful life of the antibiotic.

The present invention also has benefits for human health. Its use reduces the selection pressure for antibiotic-resistant strains of bacteria, by allowing antibiotics to be removed from animal feed. Accordingly, more antibiotic-susceptible strains will be present in the gut of the animal, thereby ensuring a more likely positive outcome in the event of antibiotics being used on the equivalent human condition.

The xylanase or cellulase enzyme to be used in the feeds can be formulated as a pre-mix together with any other enzymes to be included. The pre-mix can be added to the raw materials before feed manufacture, during feed manufacture or as a final step once the feed is otherwise

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ready for use. It is possible to add the enzyme directly as a liquid to a feed material pre-formed as pellets or as a mash.

It is also possible to include the enzyme in the animal's diet by incorporating it into a second (and different) feed or drinking water which the animal also has access to. Accordingly, it is not essential that the enzyme is incorporated into the feed itself, although such incorporation forms a particularly preferred aspect of the present invention.

If the enzyme is incorporated into a feed, then this preferably comprises at least 25% by weight of a cereal, and more preferably at least 35% by weight of the cereal. The cereal may be any one or more of wheat, maize, rye, barley, oats, triticale, rice, and sorghum. It is particularly preferred that the cereal is wheat.

Although the cereal component of a cereal-based diet constitutes a source of protein, it is usually necessary to include sources of supplementary protein in the diet, such as those derived from fishmeal, meatmeal or vegetables. These sources of supplementary protein may constitute up to 50% by weight of the animal feed. Sources of vegetable protein include at least one of full fat soybean, rapeseed, canola, soybean meal, rapeseed meal and canola meal.

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If the enzyme is incorporated into a feed, then this is preferably added in a relative amount of 0.0001-10 g of the enzyme per kilo of the feed, more preferably 0.001-1 g/kg and most preferably 0.01-0.1 g/kg.

The xylanase for use in this invention can be obtained from a fungus, such as *Trichoderma*, *Aspergillus*, *Humicola*, or *Neocallimastix*. Alternatively, the xylanase can be obtained from a bacterium, such as *Bacillus*, *Streptomyces*, *Clostridium*, or *Ruminococcus*.

The present invention is particularly effective against strains of *Salmonella* and *Campylobacter*, and especially *Salmonella enteritidis* and *Campylobacter jejuni*. Another bacterium against which the invention is effective is *Clostridium perfringens*.

Bacterial infection can be treated or prevented in accordance with the present invention in a wide variety of animals, but use of the invention is particularly preferred in domestic animals and farm livestock. Animals which may in particular benefit from the invention include poultry (such as chickens, turkeys, ducks and geese), ruminants (such as cattle, horses and sheep), swine (pigs), cats, dogs, rodents (such as rabbits) and fish. The invention is particularly useful in broiler chickens.

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The most preferred combinations of feed and enzyme include wheat plus xylanase, maize plus xylanase and barley plus  $\beta$ -glucanase.

The enzymes used in the present invention fall within a general Class called polysaccharidases. Their substrates are structural polysaccharides such as xylans and  $\beta$ -glucans that occur as an integral part of the cell wall of most land plants. These polysaccharides are not found in animal cells, and are not to be expected to have any activity against proteins. Because these enzymes attack polysaccharides found in plant cell walls, the only possible substrates for these enzymes in the gastrointestinal tract of an animal are contained in cereal-based feeds. It is therefore speculated that the beneficial effects of the xylanase or cellulase on bacterial infection result somehow from the degradation products which they produce such as xylan or  $\beta$ -glucan derived from a cereal-based diet.

As previously mentioned, WO 93/01800 discloses the use of a protease for the preparation of a medicament effective against intestinal pathogens in animals. It is well established that such pathogens mediate their infectivity by binding to receptors on the surface of intestinal epithelial cells via antigenic protein or glycoprotein molecules expressed on the pathogen's cell surface. It is suggested that the protease enzyme prevents binding of the pathogen cells to the intestinal epithelium by destroying these proteinaceous receptor/adhesion sites to

which the pathogen must bind if it is to cause an infection. The protease enzymes mentioned in this reference would be predicted to destroy proteins on the luminal surface of intestinal epithelial cells in a non-specific manner, but would not be expected to attack substrates other than proteins. Accordingly, the activity of the proteases disclosed in this reference is fundamentally different from the activity of the enzymes used in the present invention. A skilled person could not have predicted the utility of the present enzymes against bacterial infection based upon the activity of proteases disclosed in WO 93/01800.

The invention will now be described in more detail according to the following Examples.

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## Examples

### General Methodology

Wheat and maize diets were prepared having the following formulations:

Table 1 - Wheat diet

| Ingredients          | Percent |
|----------------------|---------|
| Soft Wheat           | 58.83   |
| Soybean ml 48        | 32.49   |
| Soy oil              | 4.49    |
| Salt                 | 0.30    |
| Sodium Bicarbonate   | 0.12    |
| DL Methionine        | 0.14    |
| Limestone            | 1.37    |
| Di-calcium Phosphate | 1.26    |
| Vitamins/Minerals    | 1.00    |
| TOTAL                | 100.00  |

Table 2 - Maize diet

| Ingredients          | Percent |
|----------------------|---------|
| Maize                | 55.38   |
| Soybean ml 48        | 37.30   |
| Soy oil              | 2.96    |
| Salt                 | 0.30    |
| Sodium Bicarbonate   | 0.16    |
| DL Methionine        | 0.13    |
| Limestone            | 1.22    |
| Di-calcium Phosphate | 1.55    |
| Vitamins/Minerals    | 1.00    |
| TOTAL                | 100.00  |

Animal feeds were prepared by introducing a cereal carrier containing approximately 3 mg enzyme protein/kg into the wheat diet at a concentration of 1 kg of enzyme and carrier per tonne of wheat diet. The final concentration of enzyme protein in the feed was thus approximately 3 mg per tonne. The xylanase was obtained from *Trichoderma longibrachiatum*. Broiler chicks were fed the wheat plus xylanase diet from hatching. For comparison purposes, separate flocks of chicks were fed with the wheat diet and the maize diet without the addition of xylanase. A challenge model was used, whereby

0.2 ml of a stock solution of *C. jejuni* were introduced to each chick by syringe at day 7 from hatching. The dilution level of the stock solution was varied, from  $10^{-1}$  (high challenge) to  $10^{-6}$  (low challenge, similar to natural conditions). The undiluted stock solution contained approximately  $10^4$  CFU per 0.2 ml. The caecae of the chicks were examined for damage due to the *Campylobacter* at varying ages.

#### Example 1

Figures 1-6 show the effect of the diets on *Campylobacter* colonisation in 12-day old chicks. In each case two flocks, A and B, were tested to minimise the effect of environmental variance on the results. In each case it is clearly evident that a wheat plus xylanase diet is effective in reducing the level of *Campylobacter* in the caecae of the chicks in comparison with a maize diet. Additionally, at *Campylobacter* stock solution dilution levels of  $10^{-3}$  or lower (i.e. approaching more natural conditions), the wheat plus xylanase diet becomes considerably more effective than the wheat diet alone. Thus, in Figure 1, for the wheat plus xylanase diet at a *Campylobacter* stock solution dilution of  $10^{-6}$ , a mean score of 0.5 positive caecae was observed. The equivalent scores for diets lacking xylanase were approximately 1.5 and 2.5.

### Example 2

Figures 7-9 demonstrate the effectiveness of the diets on *Campylobacter* colonisation of the small intestine and caecae of 17-day old chicks from two flocks. The effect of the wheat plus xylanase diet on reducing the *Campylobacter* colonisation of the caecae of the chicks is evident as already demonstrated in Example 1. However this reduction is even more marked as regards the small intestine. Accordingly, in Figure 7 the mean count  $\log_{10}$ CFU in the small intestine measured for chicks on the wheat and xylanase diet was less than 4. The equivalent counts for the diets not containing xylanase were found to be approximately 6, i.e. 100-fold higher.

### Example 3

Figures 10-12 depict a comparison of the effect of different diets on the weight of 1, 5, 12, 19, 25 and 33-day old chicks. Figure 10 shows the results for the wheat-based diet, Figure 11 the results for the wheat plus xylanase-based diet and Figure 12 the results for the maize-based diet. The weight of the chicks in each case is reduced by dosing with *Campylobacter*. However, those chicks to which *Campylobacter* has been introduced gain weight more quickly on the wheat plus xylanase diet than on either of the other diets.

#### Example 4

Figure 13 demonstrates the effectiveness of the wheat and wheat plus xylanase diets on *Salmonella enteritidis* colonisation of the caecae of 14-day old chicks from two flocks, A and B. The methodology employed in these experiments was identical to that employed for the *Campylobacter* experiments described above, except that the undiluted stock solution of *Salmonella enteritidis* contained approximately  $10^5$  CFU per 0.2 ml. The effect of the wheat plus xylanase diet on reducing the *Salmonella* colonisation of the caecae of the chicks is clearly evident. Thus, in flock B, the chicks on the wheat diet were found to have a mean count  $\log_{10}$ CFU/ml of approximately 7. However, the chicks from flock B on the wheat plus xylanase diet were found to have a much lower  $\log_{10}$ CFU/ml of approximately 4 (1000-fold lower).

The above Examples clearly show a reduction in bacterial infection in the gut due to the inclusion of xylanase in the diet. Similar results have been observed when using a cellulase such as a  $\beta$ -glucanase. This indicates that the use provided by the present invention significantly reduces the ability of certain bacteria to colonise the caecae which in turn prevents migration of the bacteria to the small intestine. Accordingly, since it has a reduced level of infection, the growth rate of the animal is increased, leading to economic benefits. The reduction in contamination rate also has obvious benefits to human

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health and the replacement of antibiotics by such diets  
has clear environmental benefits.

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WHAT IS CLAIMED IS:

1. A method for treating and preventing bacterial infections in an animal comprising feeding an animal a polysaccharidase enzyme in an amount effective for treating and preventing bacterial infections in the animal.
2. The method of claim 1 wherein the animal is fed the enzyme and an animal feed.
3. The method of claim 1 wherein the enzyme is mixed with an animal feed to form an enzyme/feed mixture, and the enzyme/feed mixture is fed to the animal.
4. The method of claim 1 wherein the enzyme is fed to the animal in drinking water.
5. The method of claim 2 wherein the enzyme mixture is fed to the animal along with a second animal feed.
6. The method of claims 1, 2, 3, or 4 wherein the enzyme is fed to the animal in an amount of about 0.0001 to about 10 grams of enzyme per kg of the animal feed fed to the animal.
7. The method of claim 6 wherein the enzyme is fed to the animal in an amount of about 0.001 to about 1 gram of enzyme per kg of the animal feed fed to the animal.
8. The method of claim 6 wherein the enzyme is fed to the animal in an amount of about 0.01 to about 0.1 gram of enzyme per kg of the animal feed fed to the animal.
9. The method of claim 1 wherein the enzyme is selected from the group consisting of a xylanase, cellulase, and mixtures thereof.
10. The method of claim 9 wherein the enzyme is a cellulase enzyme.

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11. The method of claim 10 wherein the enzyme is a  $\beta$ -glucanase.
12. The method of claim 1 wherein the enzyme has a form selected from the group consisting of a liquid form, a pellet, and a mash.
13. The method of claim 1 wherein the animal feed comprises at least about 25% by weight of a cereal.
14. The method of claim 13 wherein the cereal is selected from the group consisting of wheat, maize, rye, barley, oats, triticale, rice, sorghum and mixtures thereof.
15. The method of claim 14 wherein the cereal is wheat.
16. The method of claim 13 wherein the animal feed further comprises a source of protein selected from the group consisting of fishmeal, meatmeal, vegetable protein, and mixtures thereof.
17. The method of claim 9 wherein the xylanase enzyme is obtained from a fungus selected from the group consisting of *Trichoderma*, *Aspergillus*, *Humicola*, *Neocallimastix*, and mixtures thereof.
18. The method of claim 9 wherein the xylanase enzyme is obtained from a bacteria selected from the group consisting of *Bacillus*, *Streptomyces*, *Clostridium*, *Ruminococcus*, and mixtures thereof.
19. The method of claim 1 wherein the method is effective for treating and preventing bacterial infections in poultry, ruminants, swine, cats, dogs, rodents, and fish.
20. The method of claim 1 wherein the method is effective for treating and preventing bacterial infections caused by bacteria selected from the group consisting of *Salmonella enteritidis*, *Campylobacter jejuni*, *Clostridium perfringens*, and mixtures thereof.

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ABSTRACT

USE OF AN ENZYME FOR MANUFACTURING AN AGENT FOR  
CONTROLLING BACTERIAL INFECTION

Provided is the use of a xylanase or a cellulase for the manufacture of an agent for the treatment and/or prophylaxis of bacterial infection in an animal caused by *Salmonella*, *Campylobacter* or *Clostridium perfringens*.

It is preferred that xylanase is used in combination with wheat to form an animal feed. Such a diet is particularly effective in controlling *Campylobacter* and *Salmonella* in chickens.

The use provided by the present invention affords an alternative to antibiotics when controlling bacterial infection in animals. This leads to considerable health, environmental and economic benefits.

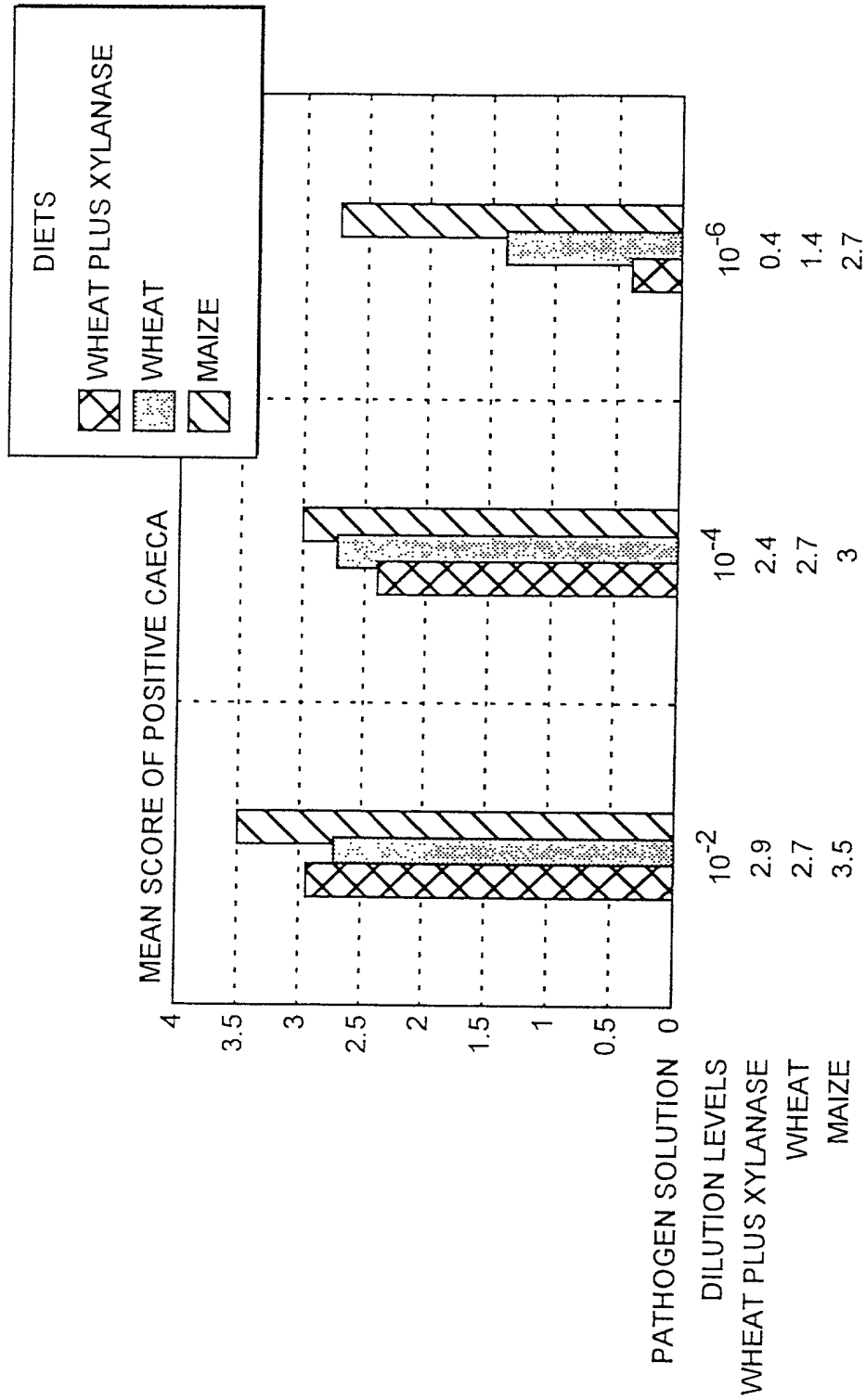


FIG. 1

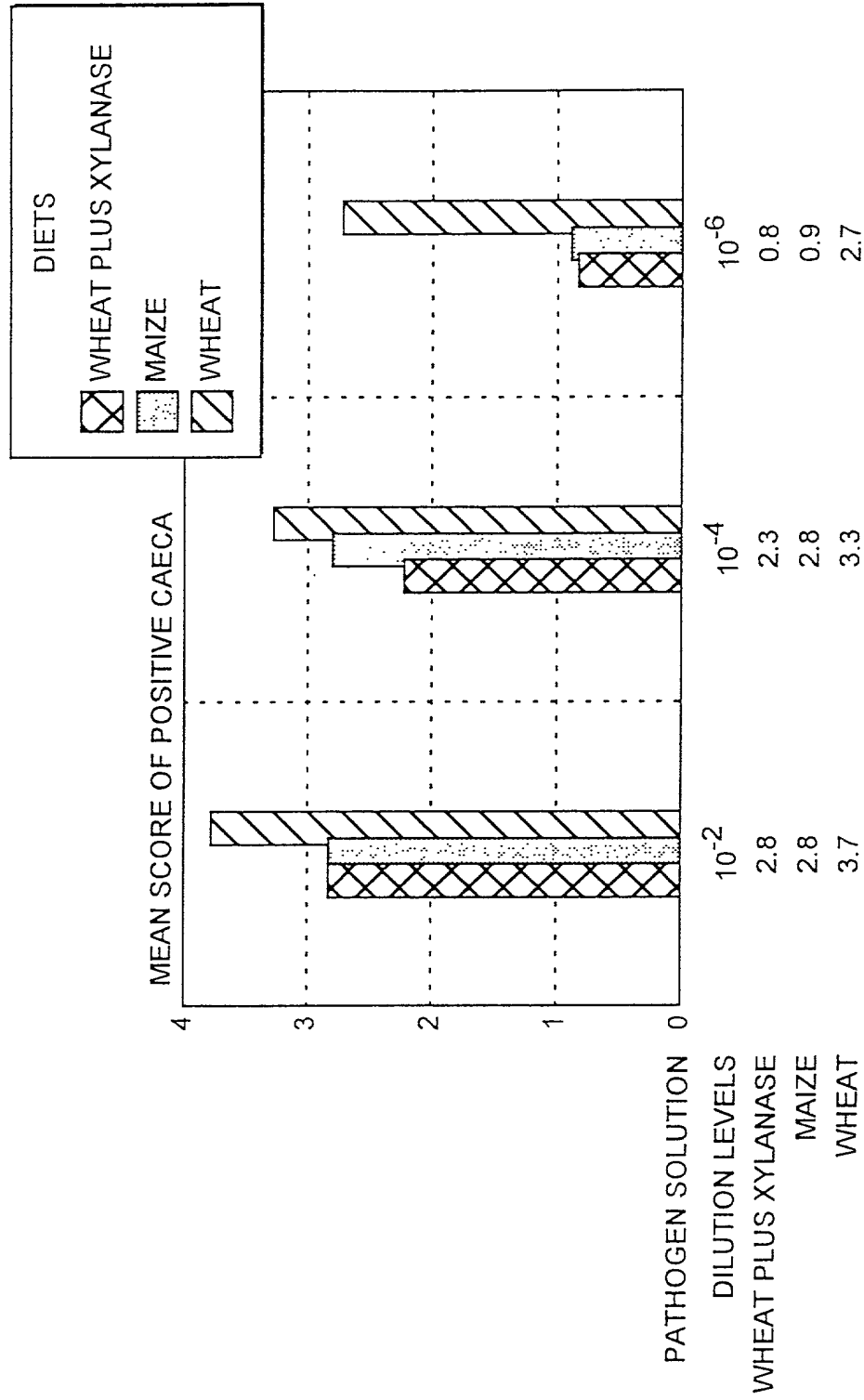


FIG. 2

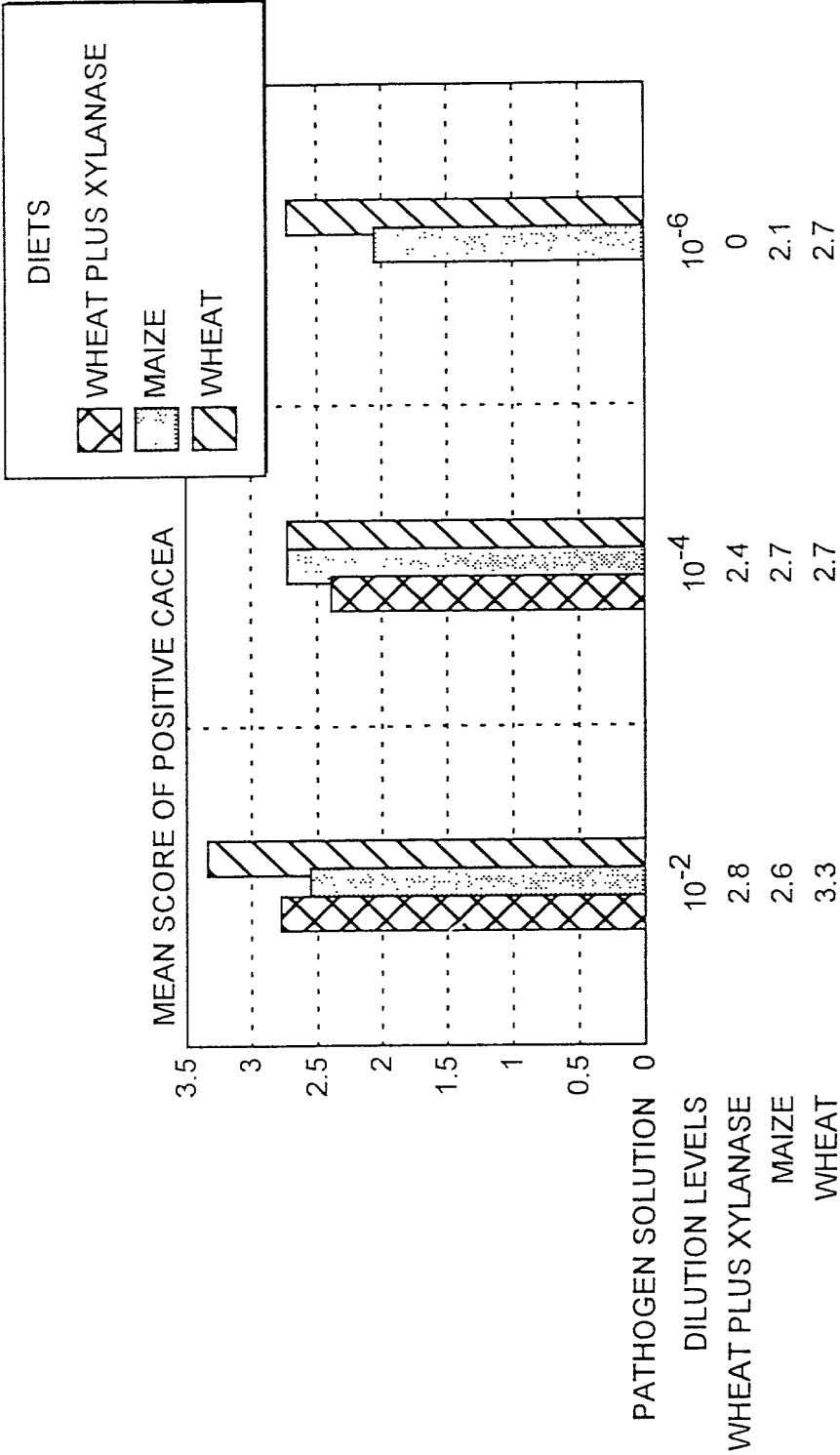


FIG. 3

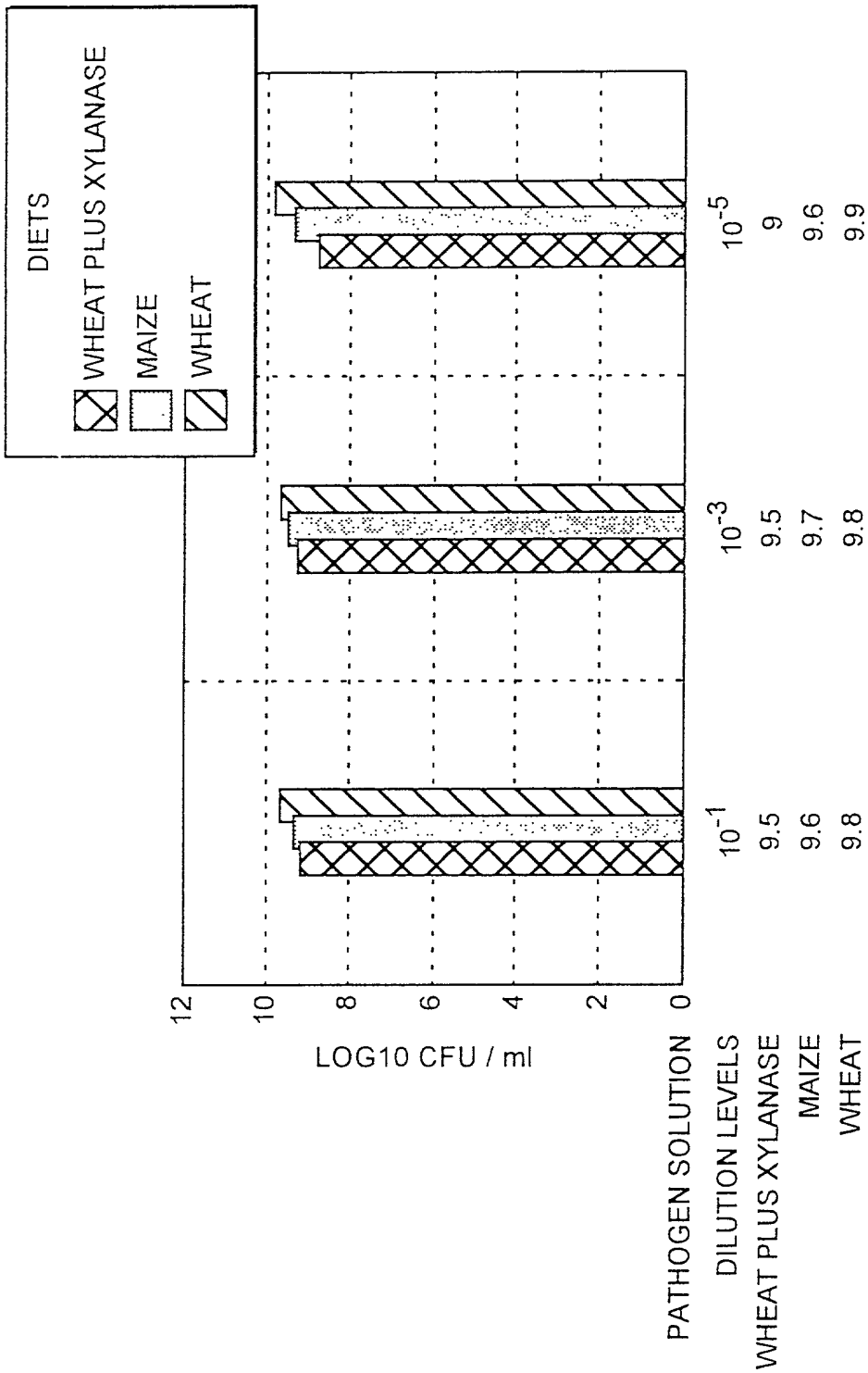


FIG. 4

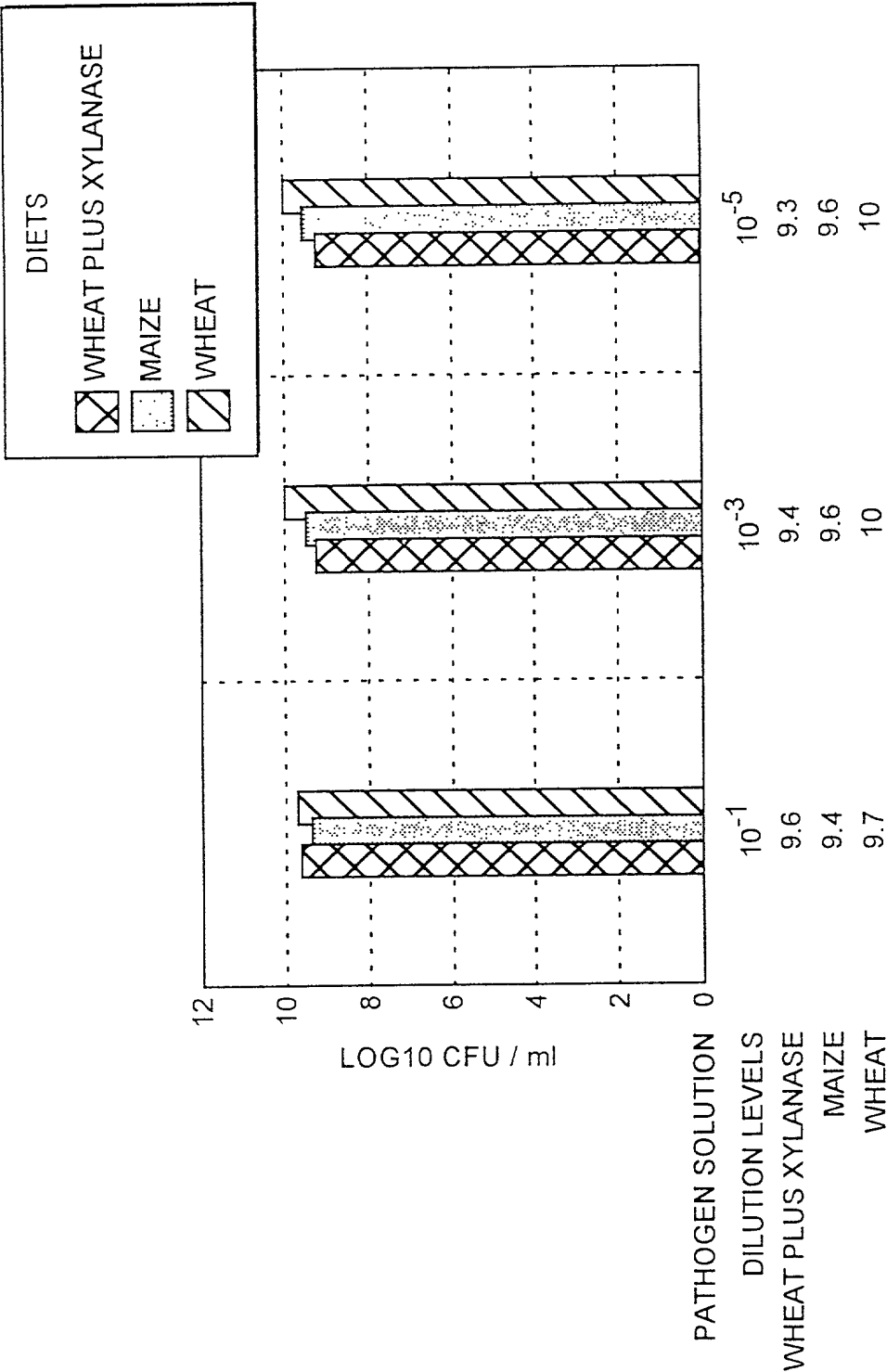


FIG. 5

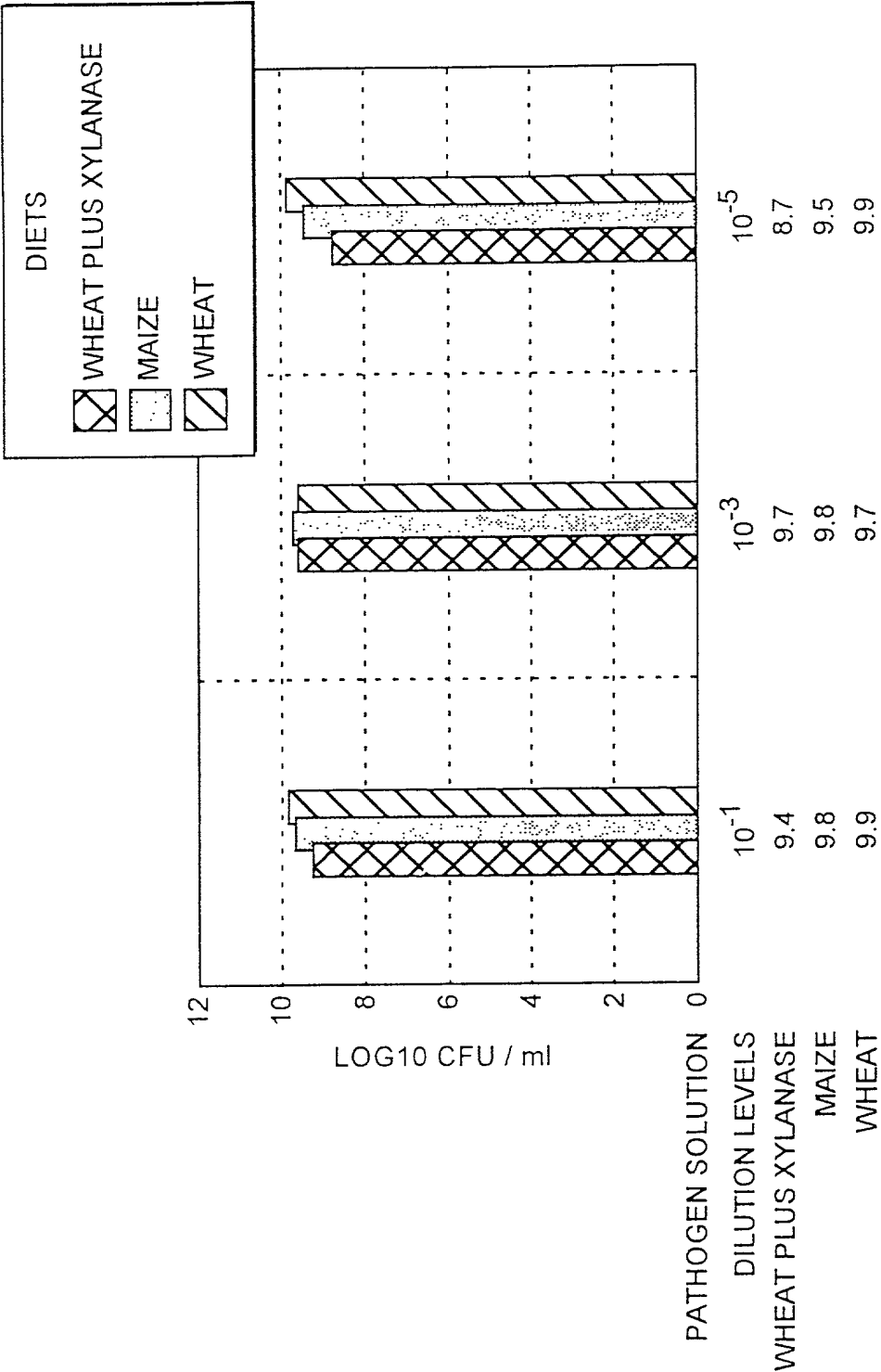
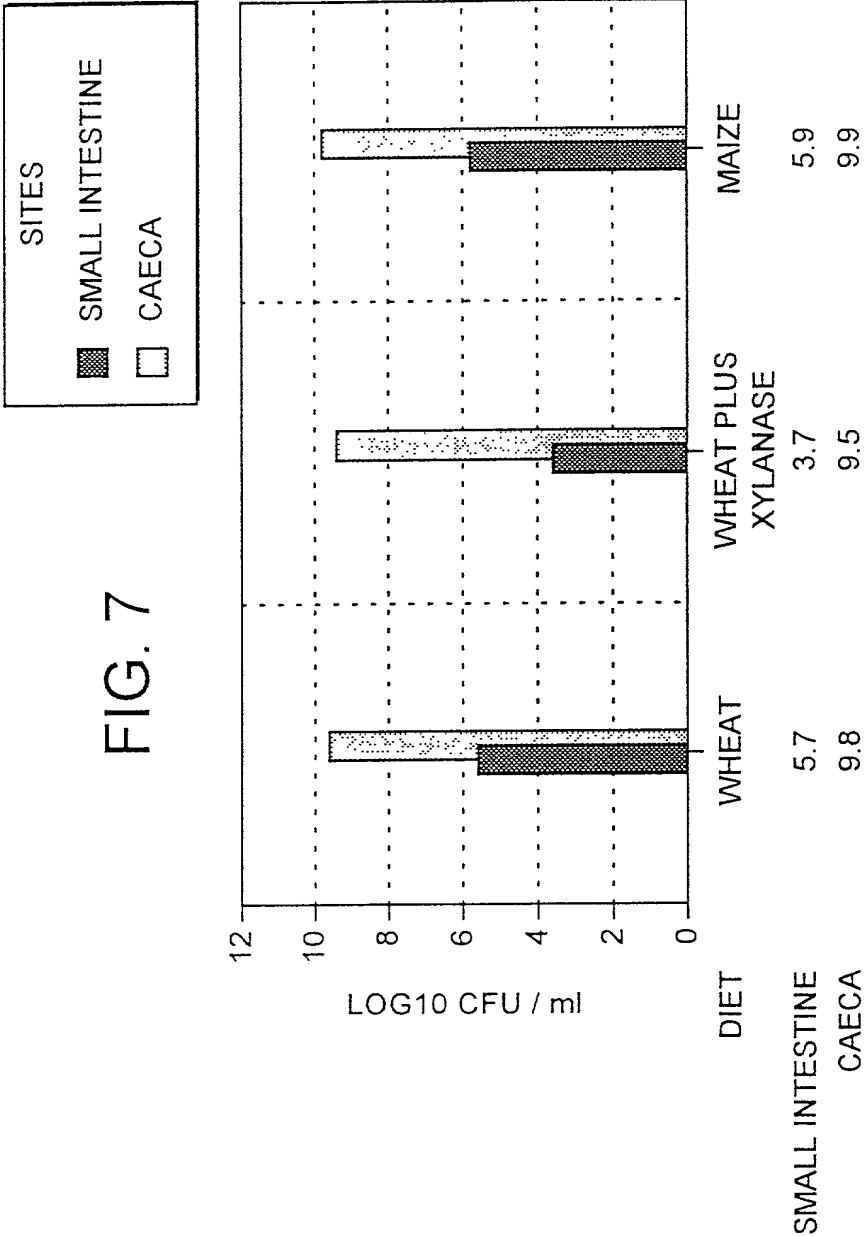
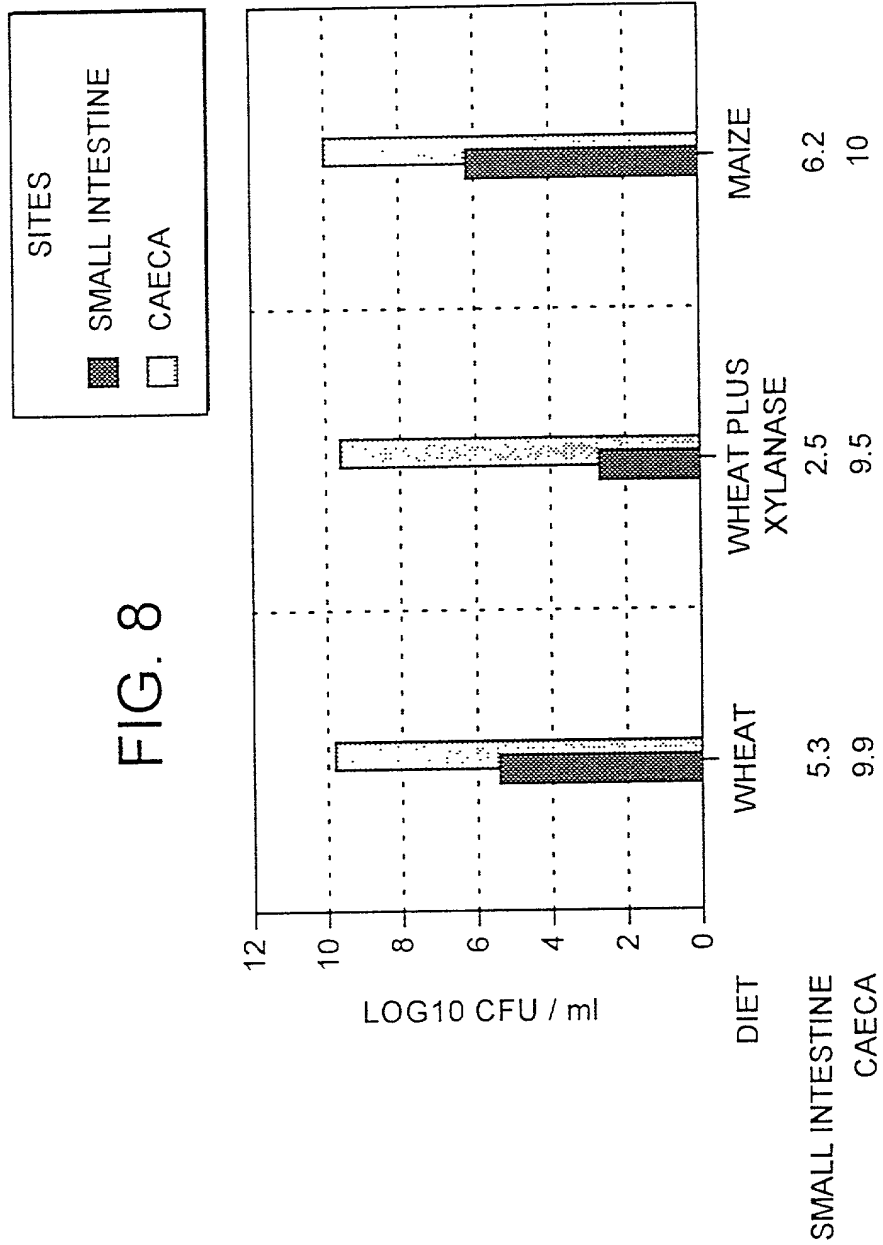


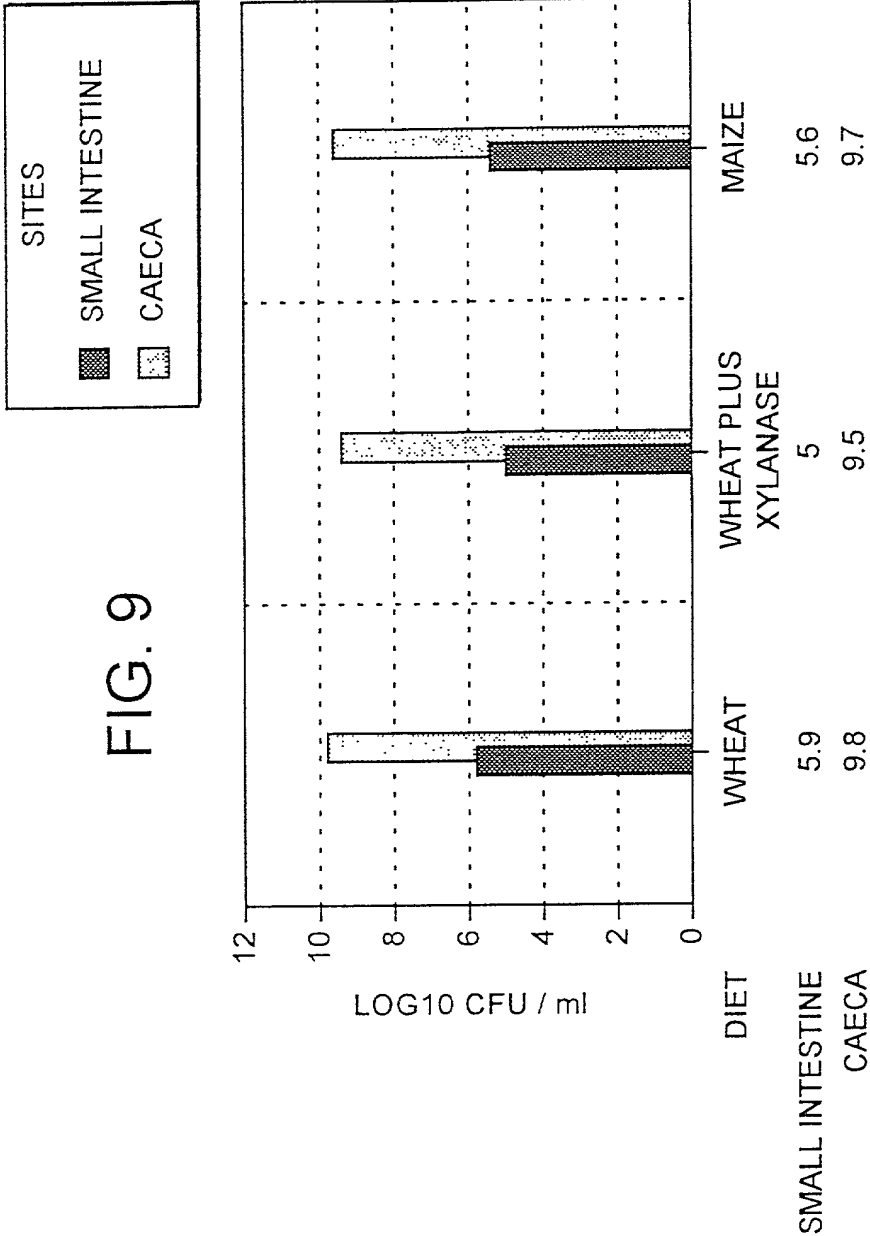
FIG. 6





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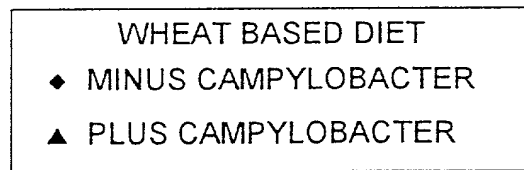
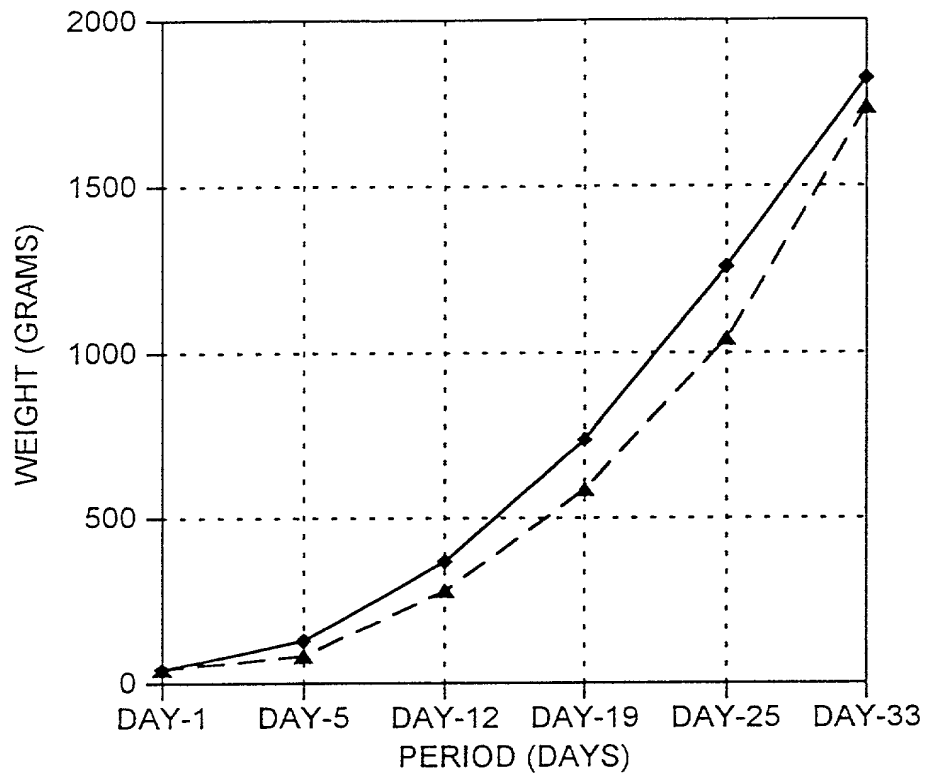


FIG. 10

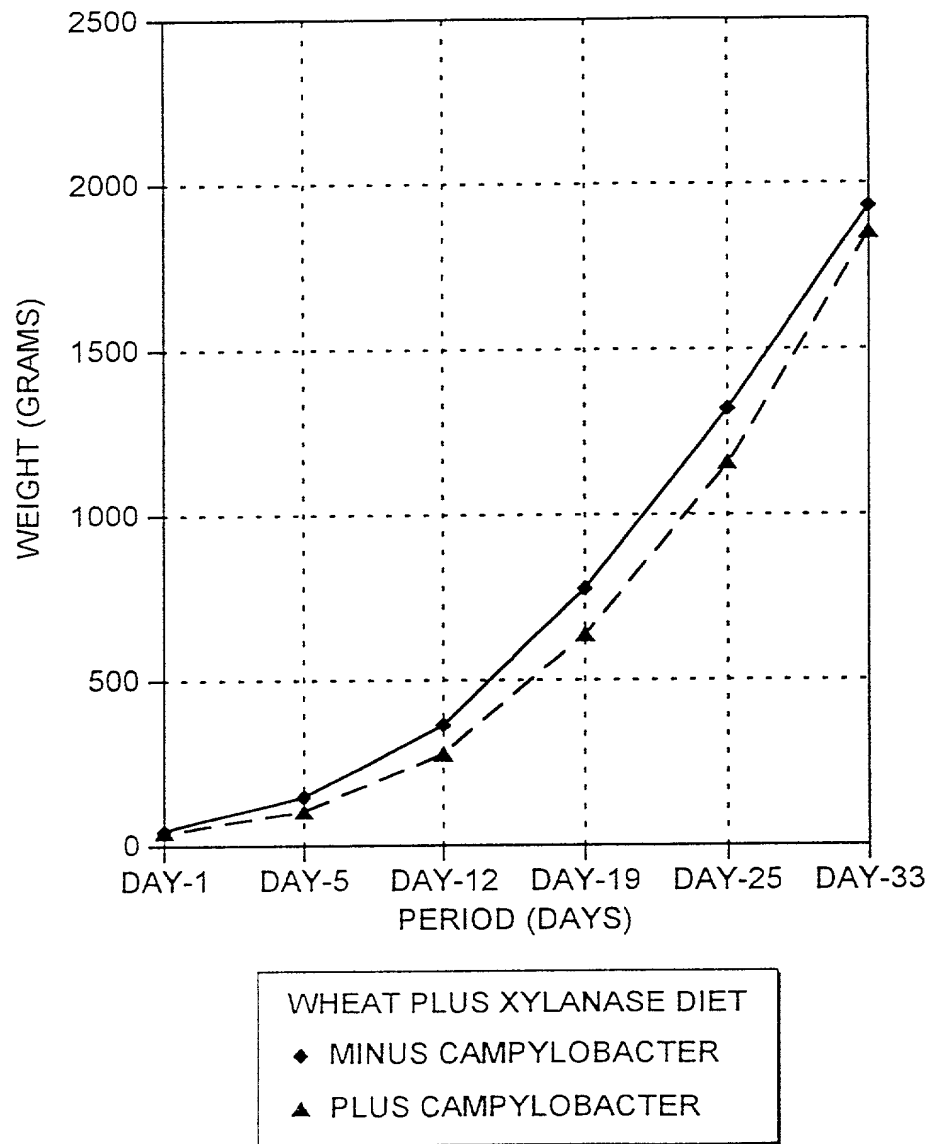


FIG. 11

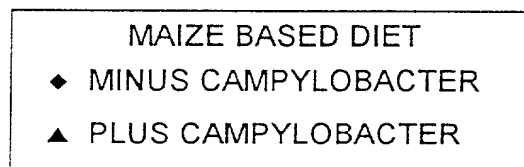
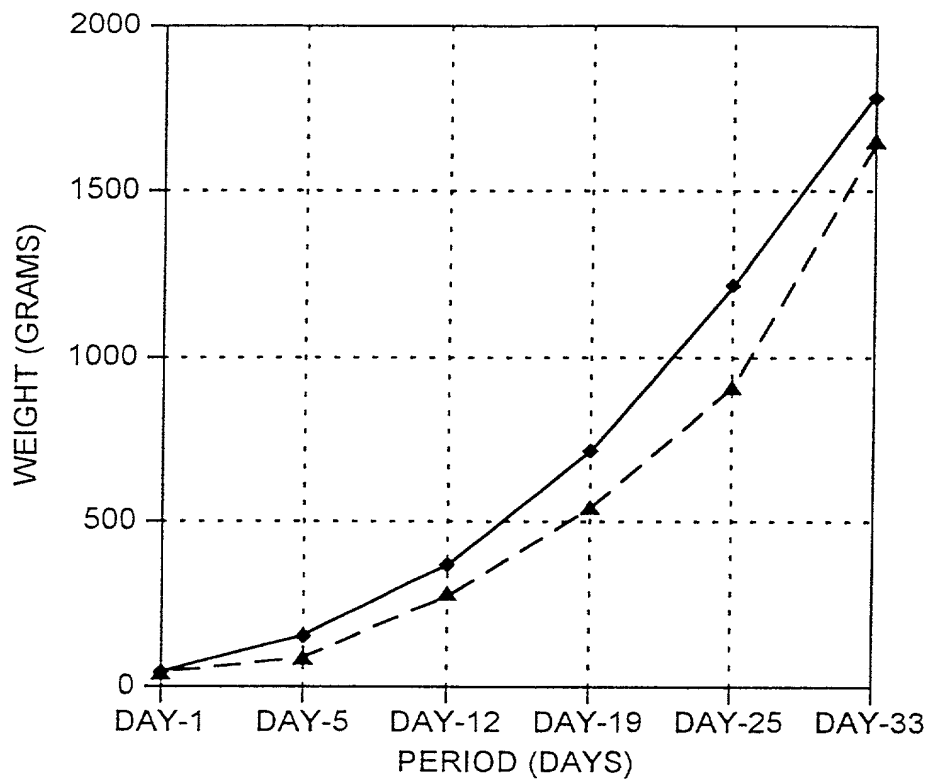


FIG. 12

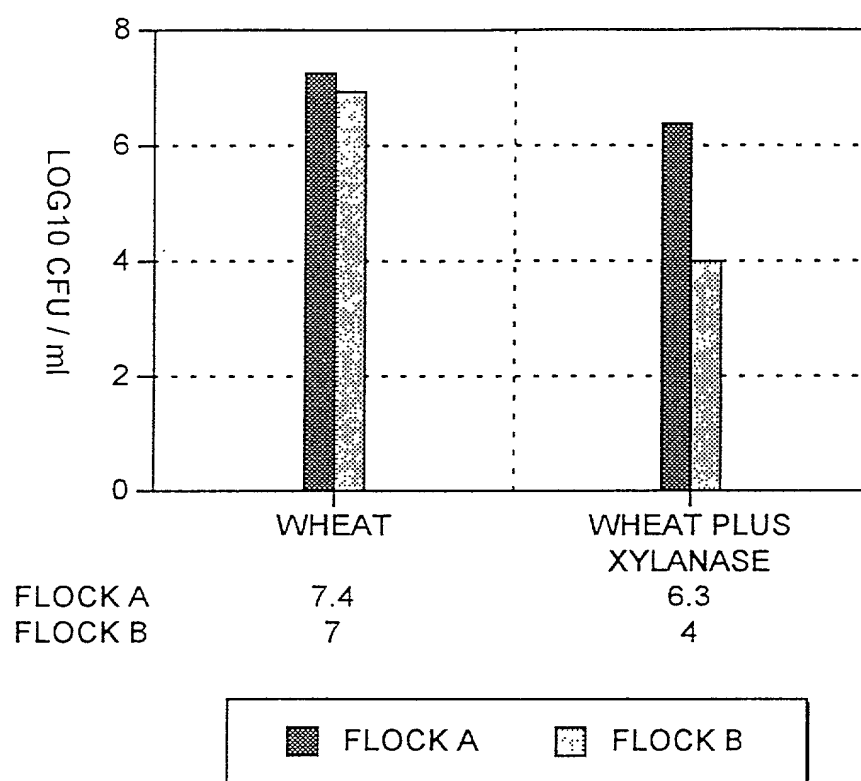


FIG. 13

**DECLARATION  
FOR UTILITY OR DESIGN  
PATENT APPLICATION**

Attorney Docket No.: 68019  
First Named Inventor: Bedford  
Application Number:  
Filing Date: Herewith  
Group Art Unit:  
Examiner Name:

X Declaration Submitted With Initial Filing  
☐ Declaration Submitted After Initial Filing

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

**USE OF ENZYME FOR THE MANUFACTURE OF AN AGENT  
FOR CONTROLLING BACTERIAL INFECTION**

(Title of Invention)

the specification of which:

(X) is attached hereto, or

( ) was filed by an authorized person on my behalf on \_\_\_\_\_ (Date)  
as United States Application Number \_\_\_\_\_  
or PCT International Application Number \_\_\_\_\_,  
and was amended on \_\_\_\_\_ (if applicable).  
(Date)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT international application which designated at least one country other than the United States of America, listed below, and I have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or any PCT international application, on this invention filed by me or my legal representatives or assigns and having a filing date before that of the application on which priority is claimed:

| Prior Foreign<br>Application<br>Number(s) | Country | Foreign<br>Filing Date | Priority<br>Not Claimed  | Certified<br>Copy Attached |                          |
|---|---------|------------------------|--------------------------|----------------------------|--------------------------|
|   |         |                        |                          | Yes                        | No                       |
|   |         |                        | <input type="checkbox"/> | <input type="checkbox"/>   | <input type="checkbox"/> |
|   |         |                        | <input type="checkbox"/> | <input type="checkbox"/>   | <input type="checkbox"/> |
|   |         |                        | <input type="checkbox"/> | <input type="checkbox"/>   | <input type="checkbox"/> |
|   |         |                        | <input type="checkbox"/> | <input type="checkbox"/>   | <input type="checkbox"/> |
|   |         |                        | <input type="checkbox"/> | <input type="checkbox"/>   | <input type="checkbox"/> |
|   |         |                        | <input type="checkbox"/> | <input type="checkbox"/>   | <input type="checkbox"/> |

☐ Additional foreign application numbers are listed on a supplemental priority data sheet attached hereto.

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below:

Provisional Application  
Number(s)

Provisional Application  
Filing Date

☐ Additional provisional application numbers are listed on a supplemental priority data sheet attached hereto.

I hereby claim the benefit under Title 35, United States Code, §120, of any prior United States application(s), or under §365(c) of any PCT international application(s) designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose all information known by me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56, which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application:

| <u>Prior U.S.</u><br><u>Application Number</u> | <u>Prior PCT</u><br><u>International</u><br><u>Application Number</u> | <u>Filing Date of</u><br><u>U.S. or PCT</u><br><u>International</u><br><u>Application</u> | <u>Patent Number</u><br><u>(if applicable)</u> |
|--|---|---|--|
|  | PCT/EP98/04440  | 16 July 1998  |  |

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority data sheet attached hereto.

As a named inventor, I hereby appoint the practitioners associated with Customer Number 22242, with full power of substitution and revocation, to prosecute this application and to transact all business in the United States Patent and Trademark Office connected therewith, and request that all correspondence and telephone calls in respect to this application be directed to FITCH, EVEN, TABIN & FLANNERY, Suite 1600, 120 South LaSalle



Street, Chicago, Illinois 60603-3406, Telephone No. (312) 577-7000,  
Facsimile No. (312) 577-7007, CUSTOMER NUMBER 22242.



I hereby declare that all statements made herein of my own knowledge are true, and that all statements made herein on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity or enforceability of the application or any patent issued thereon.

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Great Britian